The association of PCOS and hypertensive disorders of pregnancy -a community based approach

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Abstract

OBJECTIVE: To investigate the prevalence of hypertensive disorders of pregnancy (HDP), and the respective roles of PCOS, obesity, lifelong weight gain (WG) and hyperandrogenemia in the disease development by age 46.

METHODS: The Northern Finland Birth Cohort 1966, with follow-up at ages 14, 31 and 46, including women with (n=408) and without (n=3373) HDP diagnosis. HDP diagnosis was combined from the questionnaire data at age 46, hospital discharge records and Finnish Medical Birth Registers. Women with both oligo-amenorrhea and hirsutism at age 31 or with PCOS diagnosis by age 46 (n=279) were compared with the women without PCOS (n=1577).

RESULTS: Women with PCOS had an increased HDP risk (odds ratio [OR]=1.56 [95%CI:1.03-2.37]), but the association disappeared after adjusting for BMI at age 31. The risk for HDP was not increased among normal weight PCOS women. The increase of BMI (kg/m^2) from age 14 to 31 was significantly greater in both PCOS (median (interquartile range): 5.94(3.69;11.1), $p<0.001$) and non-PCOS (4.89(3.21;7.57), $p<0.001$) women with HDP and also in PCOS women without HDP (4.59(2.40;7.54), $p=0.009$) compared to non-PCOS without HDP. Among women with PCOS, BMI increase was greater in women with than without HDP (5.94(3.69;11.1) vs 4.59(2.40;7.54), $p=0.015$). Hyperandrogenemia at ages 31 or 46 did not associate with HDP (OR=1.44 [95%CI: 0.98-2.11]).

CONCLUSION: Obesity and weight gain from adolescence to age 46, but not PCOS *per se* or hyperandrogenemia, were associated with an increased risk of HDP. There was a strong synergistic association of PCOS and obesity regarding HDP risk.

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting 5 to 15% of women at fertile age (Archer, Chang, 2004, Franks, 1995, Kjerulff, Sanchez-Ramos & Duffy, 2011). PCOS is defined by the presence of two of the following criteria: (i) polycystic ovaries (PCO); (ii) oligo-amenorrhea (OA) or amenorrhea; and/or (iii) clinical or biochemical evidence of hyperandrogenism (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004, Teede, Missio et al. 2019). The syndrome is associated with overweight and obesity in 20% to 80% of the women depending on the studied population and diagnostic criteria used (Sam, 2007, Lim et al., 2019). Importantly, insulin resistance is a pivotal disorder in PCOS, affecting approximately 25% to 70% of the women with the syndrome and is exacerbated by overweight and obesity (Diamanti-Kandarakis, 2007, Sam, 2007).

Hypertensive disorders of pregnancy (HDP) complicate 5-10% of all pregnancies (Hashemi et al., 2013). Hypertensive disorders of pregnancy include chronic hypertension (blood pressure of at least >140/90 mmHg before pregnancy or before 20 weeks of gestation), preeclampsia (new onset of hypertension and proteinuria after 20 weeks of gestation), superimposed preeclampsia (chronic hypertension in association with preeclampsia) and gestational hypertension (defined according to the same criteria but without proteinuria) (Wang et al., 2013, Davison et al., 2004a (American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy 2013)). All these disorders are associated with increased maternal and fetal morbidity and mortality during pregnancy and can also affect the future health of both the mother and child (Mannisto et al., 2013). Women with a history of HDP seem to be at higher risk of chronic hypertension, dyslipidemia, cardiovascular diseases (CVDs), type 2 diabetes mellitus and kidney disease in later life (Hashemi et al., 2013, Mannisto et al., 2013). HDP, especially pre-eclampsia, predisposes fetus to intrauterine growth restriction and these

children are also reported to have an increased risk for CVDs later in life. (Davison et al., 2004b, (Herrera-Garcia, Contag 2014)).

Recent meta-analyses have suggested that PCOS *per se* is associated with an increased risk of pregnancy induced hypertension (PIH) and preeclampsia ((Bahri Khomami, Joham et al. 2019), Boomsma et al., 2006, Qin et al., 2013, Kjerulff, Sanchez-Ramos & Duffy, 2011), although conflicting results have also been obtained (Altieri et al., 2010, Haakova et al., 2003, Mikola et al., 2001). Of note, in some studies, the association between PCOS and HDP has been confounded by multiple factors such as a higher multiple pregnancy rate, parity, age and body mass indexes (BMI) (Boomsma et al., 2006, Mumm et al., 2015). It has also been suggested that the increased risk of HDPs in PCOS may be linked mainly to obesity or hyperandrogenemia, but not specifically to the syndrome itself.

This study has two main aims: firstly, to investigate in a population-based follow-up cohort study, whether women with PCOS experience an increased prevalence of HDP during their reproductive life. Secondly, to identify the impact of factors associated with PCOS, particularly obesity and hyperandrogenemia, on the development of HDP. More specifically, we wanted to explore the significance of weight gain from adolescence to adulthood regarding the emergence of HDP both in PCOS and in non-PCOS women.

METHODS

Data collection and study population

The study population consisted of the Northern Finland Birth Cohort 1966 (NFBC66), a unique population-based, follow-up cohort of subjects (12058 born alive during 1966 in two northernmost provinces of Finland, of these 5889 females). Collection of this database began at the 24th gestational week and was supplemented by data collected at ages 14, 31 and 46. At age 14, in 1990, the adolescent females (n=5455, 94.6%) answered a postal questionnaire, with

the help of their parents, including questions about weight and height. In 1997, at age 31, a postal questionnaire, including questions about health, behavior, work and social background, was sent to 5608 women and 4523 (81%) of them responded. In addition, those living in Northern Finland or in the Helsinki metropolitan area (n=4074) were invited to a clinical examination. Of these, 3127 (77%) women participated in a clinical examination including anthropometric measurements and blood samples for hormonal and metabolic parameters. Again, at age 46, a new large questionnaire including all main health issues and an invitation to clinical examination was sent to 5123 women. Of these, 3706 (72.3%) answered the questionnaire and 3280 women (64.0%) participated in the clinical examinations, including also blood samples. (Figure 1). When gathering the final study population women without deliveries were excluded from the analyses.

In all clinical examinations, participants' weight (kg) was measured with a regularly calibrated, digital scale. Height (cm) was measured twice by using standard and calibrated stadiometer and the average of the measurements was calculated. Body mass index (BMI) was calculated (kg/m^2) and women who were overweight (BMI ≥25 kg/m^2) or obese (BMI >30 kg/m^2) were identified. BMI values at ages 31 and 46 from clinical examination and postal questionnaire were combined to create a variable where clinically measured BMI was primarily used and self-reported BMI used if measured BMI was not available. The clinically measured and self-reported BMIs did not differ (Ollila, Piltonen et al. 2016, Koivuaho, Laru et al. 2019). Weight changes (median ±SD) between ages 14-31, 31-46 and 14-46 as well as increase in waist circumference between ages 31 and 46 were calculated in each study group.

Definition of HDP diagnosis

The diagnosis of HDP had to be assessed at least in two of the three following sources to be considered as reliable: the Finnish Medical Birth Register (FMBR), the hospital discharge

register (HDR) or the questionnaire at age 46. The process is described in more detail in Supplemental Figure 1.

Data on women´s pregnancies and deliveries until the end of 2013 was obtained from the FMBR. The FMBR, active since 1987, is currently run by the National Institute for Health and Welfare. For each delivery in Finland, a structured form for FMBR is completed by the delivery hospital, including demographic and health data of the mother, the course and complications of the pregnancy (including HDP diagnosis) and the delivery, and the perinatal health of the newborn until the age of seven days. The FMBR is supplemented with data compiled by the Population Register Centre on live births and by Statistics Finland on stillbirths and deaths during the first week of life. After these additions, the registration of birth is 100%.

The HDR was checked for the data available (years 1972-2017) and the ICD-8, ICD-9 and ICD-10 diagnostic codes for HDPs were identified.

Questions about HPD (chronic hypertension, PIH and preeclampsia) in the 46-years questionnaire were asked as follows: If you have been pregnant, have you been diagnosed during pregnancy with 1) hypertension (including preexisting chronic hypertension and PIH) 2) hypertension and proteinuria (=pre-eclampsia)?

According to the questionnaire, 665 women were diagnosed with HDP by age 46. Of those women, 358 were not given a formal diagnosis of HDP to the HDR or the FMBR. Their medical records were therefore checked, and for 51 of those 358 the diagnosis of HDP was confirmed in the patients' records. The remaining women whose diagnosis could not be confirmed were excluded from further analyses (n=307). After the exclusion of the women with a diagnosis of HDP from only one source (n=522), the final study group therefore comprised 408 women with a confirmed diagnosis of HDP. The women without diagnosis of HDP from any of these three sources were considered as control women (n=3373, Supplemental Figure 1).

166 ***Definition of PCOS diagnosis***

167 At age 31, the questionnaire included two questions on hirsutism (H) and oligo-
168 amenorrhea (OA): 1) is your menstruation cycle over twice a year more than 35 days? and
169 2) do you have excessive body hair? Of the women who responded to the questionnaire
170 (excluding women using hormonal contraception, n=1459 and not permitting the use of
171 their data for data analysis, n=41), 10.4% (n=321) reported isolated H, 10.2% (n=330)
172 isolated OA and 3.4% (n=125) both OA and H (Koivunen et al., 2008, Rantakallio, 1988,
173 Taponen et al., 2004). Women with only one PCOS symptom were excluded from the
174 analyses. At age 46, the question on self-reported PCOS was inquired as follows: Have you
175 been diagnosed with polycystic ovaries (PCO) and/or PCOS? Women with either both
176 symptoms at age 31 and/or self-reported PCO/PCOS diagnosis by age 46 were classified
177 as cases (n=279), which is consistent with both the National Institutes of Health and the
178 Rotterdam criteria for diagnosis of PCOS (Rotterdam ESHRE/ASRM-Sponsored PCOS
179 Consensus Workshop Group, 2004, ZAWADSKI JK, 1992). Women without any PCOS
180 symptoms at age 31 and without self-reported diagnosis of PCOS by age 46 were classified
181 as "non-PCOS controls" (N=1577, Figure 1).

182 ***Final study population***

183 The study population was further divided into four groups: women with PCOS with HDP
184 (n=36), women with PCOS without HDP (n=154), non-PCOS women with HPD (n=161) and
185 non-PCOS women without HDP (n=1045) (Figure 1).

186 ***Laboratory methods***

187 Biochemical assays and laboratory methods of the clinical examination (at age 31) are detailed
188 elsewhere (Taponen et al., 2003). Sex hormone binding globulin (SHBG) at the age of 46 years
189 was analyzed by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare,
190 Llanberis, UK). Analytical sensitivity of method was 0.02 nmol/l. The intra-assay coefficients

of variation (CVs) of the method were 4.5% and 9.7% for concentrations 5.6 and 89.3 nmol/l, respectively. The inter-assay CVs of the method were 4.1% and 5.3% for concentrations 8.4 and 38.5 nmol/L, respectively. Serum samples for assay of total testosterone (T) at ages 31 and 46 were analyzed by using Agilent triple quadrupole 6410 LC-MS equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies, Wilmington, DE, USA). Multiple reaction monitoring was used to quantify T by using trideuterated T (d3-T), with the following transitions: m/z 289.2 to 97 and 289.2 to 109 for T and 292.2 to 97 and 292.2 to 109 for d3-T. The intra-assay CVs of the method were 5.3%, 1.6% and 1.2% for T at 0.6, 6.6 and 27.7 nmol/l, respectively.

A woman was considered as having an elevated T levels if T was over 2.3 nmol/l at age 31 (upper limit in our accredited laboratory in fertile age women) or over 1.7 nmol/l at age 46. The cut-off value for T at age 46 was determined according to the upper limit of 97.5% reference range in non-PCOS women in the study population. The free androgen index (FAI) was calculated by using the equation 100×T (nmol/l)/SHBG (nmol/l).

Statistical methods

The differences in distributions of clinical characteristics were tested by using nonparametric Mann-Whitney U test, when appropriate, otherwise a *t*-test was used. The p-values were further adjusted for BMI at ages 31 and 46 using univariate general linear modelling (ANCOVA). Categorical data were analyzed using cross-tabulation and Pearson's Chi-squared (χ^2) test. Continuous data are presented as medians with lower (25^{th}) and upper quartiles (75^{th}) (interquartile range, IQR).

The whole study population was also stratified into quartiles regarding serum total T level and free androgen index (FAI) at age 31 and 46. Chi-squared (χ^2) test's Linear-by-Linear association was used to identify the trend of HDP prevalence across these quartiles. The p-values were further adjusted for BMI at age 31 and 46 using a binary logistic regression model.

Binary logistic regression models were employed to estimate the factors associated with HDP. Several covariates were also included into the models, including consumption of alcohol, smoking and education status at age 46. The results are reported as odds ratios (ORs) with 95% confidence intervals (95% CIs). A p-value <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA).

Ethical approval

The Ethics Committee of the Northern Ostrobothnia Hospital District approved the study and written informed consent was obtained from all subjects. All subjects received written and oral information and gave their written consent to use all data.

RESULTS

Association of PCOS with HDP

The prevalence of HDP was 14.1% (n=197/1396) in the whole population. Women with PCOS presented more often HDP compared with the non-PCOS controls 18.9% vs 13.3%, respectively, (p=0.044, Figure 2) and displayed also a slightly increased risk of HDP (OR=1.56 [95% CI: 1.03-2.37]). The risk between the groups, however, was abolished after adjustment for BMI at age 31. (Figure 3A). The results were similar when replacing BMI at age 31 by BMI at age 46 or by waist circumference at age 31 or 46 (data not shown). When obese (BMI ≥30 kg/m^2 vs BMI≤30) women with PCOS were compared with the non-PCOS controls, the association with HDP was significant in obese PCOS women at ages 31 (OR=6.40 [95%CI:3.10-13.23]) (Figure 3A).

Comparison of women with PCOS with and without HDP (Table 1, Figures 4A and 4B)

When comparing women with PCOS with or without HDP, BMI was significantly higher at ages 14 (p=0.022), 31 (p=0.001) and 46 (p<0.001) and waist circumference was significantly

241 greater at age 46 (p=0.003) in PCOS cases with HDP. They also had greater weight gain from
242 adolescence to late adulthood (Table 1, Figure 4A). However, the increase of waist
243 circumference (age 31-46) did not significantly differ between the two groups (Figure 4B). At
244 ages 31 and 46, FAI was significantly higher in women with PCOS and HDP (p=0.015 and
245 p=0.020, respectively), but statistical significance was lost after adjustment for BMI.
246
247 ***Comparison of women with HDP with and without PCOS (Table 1 and Figures 4A and 4B)***
248 When comparing HDP women with PCOS to those without PCOS, BMI was significantly
249 higher in the PCOS group at ages 14 (p=0.018), 31 (p<0.001), and 46 (p=0.003) and their weight
250 gain was greater from age 14 to 31 (p=0.033). (Table 1 and Figure 4A). Waist circumference
251 was significantly greater at ages 31 (p=0.021) and 46 (p=0.011). The increase of waist
252 circumference between ages 31 and 46 did not differ and the pattern of this increase was very
253 similar between the two groups (Figure 4B).
254 Further, in HDP women with PCOS, the serum levels of T were significantly
255 higher (p=0.011) at age 31, and FAI was significantly higher at age 31 (p=0.002) and 46
256 (p=0.007) than in HDP women without PCOS. After BMI adjustments, statistical significance
257 was lost regarding serum levels of T (p=0.061) but the difference in FAI remained significant
258 at both ages 31 (P=0.012) and 46 (p=0.037).
259 ***Comparison of non-PCOS women with and without HDP (Table 1, Figure 3B)***
260 In the non-PCOS women with HDP, BMI was significantly higher at ages 14 (p=0.011), 31
261 (p<0.001) and 46 (p<0.001), and waist circumference was significantly greater at ages 31
262 (p=0.005) and 46 (p<0.001) compared to the non-PCOS women without HDP. Weight gain was
263 significantly greater between ages 14-31 (p<0.001) and 14-46 (p<0.001) in the HDP group. In
264 the non-PCOS women at age 31, the risk of HDP increased along with BMI class (Table 1,
265 Figure 3B).

Association of hyperandrogenemia with HDP in the whole population

In the whole population, women with elevated serum T (>2.3nmol/l at age 31 or >1.7nmol/l at age 46) did not have a significantly greater risk of HDP compared to non-hyperandrogenic controls. The levels of FAI expressed as medians were significantly higher at ages 31 (4.53 [2.62; 7.63] vs. 3.76 [2.43; 5.72], p=0.009) and 46 (1.72 [1.25; 2.46] vs. 1.52 [1.06; 2.16], p<0.001) in women with HDP compared with women without HDP and the significance remained at age 46 after adjustment for BMI (p=0.042). The prevalence of HDP in the T quartiles was not significantly linearly associated at age 31 (p=0.337) or at age 46 (p=0.895). In the FAI quartiles, the prevalence of HDP were significantly linearly associated at age 31 (p=0.019), and at age 46 (p<0.001), but overall significance was lost after adjustment for BMI (Supplemental Figure 2).

Discussion

This large follow-up, cohort study indicates that the increased risk of HDP in PCOS can mostly be attributed to overweight or obesity and that normal weight women with PCOS seem not to be at increased risk for developing HDP. More specifically, our study revealed also that weight gain from adolescence until the end of reproductive life was the most significant parameter associated with HDP both in PCOS women and in non-PCOS women. Lastly, our results could not confirm any significant association of hyperandrogenemia with the development of HDP.

Comparison with other studies

The total prevalence of HDP was 14.1% in our study population, which is relatively high when compared to other studies - typically reported a prevalence of HDP are between 5 and 10% (Hutcheon, Lisonkova & Joseph, 2011, Hashemi et al., 2013, Umesawa, Kobashi, 2017). It is of note, however, that direct comparison of HDP prevalence across populations from different countries is challenging, given the considerable heterogeneity in screening approaches,

diagnostic criteria, and underlying population characteristics. Moreover, it seems that the prevalence of HDP is increasing widely due to the rising burden of obesity in women of reproductive age (Hutcheon, Lisonkova & Joseph, 2011).

In the whole group of women with PCOS, the risk of HDP was significantly increased but in overweight/obese women with PCOS the risk was more than three-fold compared to normal weight non-PCOS women. These findings are in line with the conclusions of recent meta-analyses, suggesting that women with PCOS have an increased risk of developing PIH and preeclampsia (Bahri Khomami, Joham et al. 2019), Boomsma et al., 2006, Qin et al., 2013, Kjerulff, Sanchez-Ramos & Duffy, 2011). In the present study, however, the difference disappeared when comparing overweight/obese PCOS women with overweight/obese non-PCOS women, and the prevalence of HDP was not increased among normal-weight PCOS women compared to their normal weight non-PCOS counterparts. Similar results were found in the non-PCOS groups when comparing the risk of HDP in obese or overweight with their normal weight counterparts, suggesting that the risk of HDP is mostly attributable to overweight/obesity, in line with some earlier data (Ye et al., 2014, Fuchs et al., 2017). The diverging conclusions of the aforementioned meta-analyses could be explained by the fact that the majority of the eligible studies either did not take into account confounding factors such as BMI, were of retrospective study design and included a relatively small sample size. Only few previous studies have addressed the possible interaction of BMI with PCOS regarding the risk of HDP. Lonnebotn et al also reported an increased risk of HDP in obese women with PCOS but not in normal weight or overweight women with PCOS (Lonnebotn et al., 2018). However, that study reported also increased risk in underweight (BMI <18.5 kg/m^2) women, whereas there were no underweight women with both PCOS and HDP in our study. All in all, the results of the present study indicate that increased BMI significantly enhances the risk of HDP and that PCOS seems to synergistically increase this risk.

In previous literature, there is a lack of long-term follow up studies investigating the association of lifelong increase of weight or of waist circumference with the risk of HDP. In this study, the women with HDP (PCOS and non-PCOS) experienced a significantly greater increase in weight from adolescence to late adulthood compared with women without HDP (both PCOS and non-PCOS, respectively). Interestingly, the pattern of increase in waist circumference between ages 31 and 46 was mostly associated with HDP, but not with PCOS status. These findings further strengthen the role abdominal obesity and insulin resistance, as a pivotal factor associated with the development of HDP. The existence of a complex synergistic interrelationship between PCOS, abdominal obesity and weight gain are most likely risk factors for these alterations.

In the whole study population, the risk of HDP was not significantly increased in the group of women with elevated serum T levels compared with controls. However, hyperandrogenemia assessed by FAI was significantly associated with HDP, and both PCOS and non-PCOS women with HDP had higher values of FAI compared with non-PCOS women without HDP. Again, after adjusting for BMI, hyperandrogenemia lost its statistical significance. Some previous studies have suggested that the hyperandrogenic PCOS phenotypes are associated with higher prevalence of HDP, especially preeclampsia, compared to normoandrogenic phenotypes (Palomba et al., 2010, Naver et al., 2014) but other studies have produced conflicting results (Mumm, Jensen et al. 2015). Hyperandogenism has been associated with preeclampsia also in the absence of PCOS (Carlsen, Romundstad et al. 2005, Perez-Sepulveda, Monteiro et al. 2015, Ghorashi, Sheikhvatan 2008, Hakim, Padmanabhan et al. 2017). A possible explanation for these observations may be that placental aromatase (the enzyme responsible for the conversion of androgens to estrogens) is deficient in placental ischemia and preeclamptic pregnancy (Perez-Sepulveda et al. 2017), thus explaining the observed elevation of maternal androgens during preeclampsia. Elevated androgens have also

been postulated to play an important role in the etiology of preeclampsia, although the mechanism is not clear (Gorashi and Sheikhvatan 2008). In the present study, the lack of association between hyperandrogenemia and HDP may be partly due to the fact that we were not able to differentiate the diagnosis of pre-eclampsia from the other causes of HDP. Based on the present and those earlier results, the role of hyperandrogenemia in the pathogenesis of HDP remains therefore under debate and needs further research to be clarified.

Strengths and limitations

The main strength of this study is the prospective population-based cohort design with the longest follow-up time compared with previous studies investigating the relationship between BMI and HDP in women with and without PCOS. All subjects were of Caucasian ethnicity and living in the same area during the same time period. The participation rates for the clinical examinations and questionnaires at ages 31 and 46 were remarkably high and anthropometric parameters were mostly clinically measured. In Finland, free of charge, mandatory follow-up of pregnancy in health care centers and practically free-of-charge maternal care in public hospitals is offered equally to all pregnant women, which enabled us to perform data collection on pregnancy complications in virtually all women participating in the 46-year questionnaire. By taking advantage of FMBR, HDR and the questionnaire data we were able to have an accurate estimate of HDP in our study population as HDP diagnosis was set only if it was found in at least two out of the three sources. Moreover, our study included careful adjustments for possible confounders allowing us to identify the respective effects of PCOS *per se* and other risk factors, such as obesity, hyperandrogenemia and weight gain from adolescence to late adulthood, on risk of having developed HDP during reproductive life. Lastly, the measurements of total T were performed by using LC-MS equipment, which has been considered as the "golden standard" for measuring total T in women.

A potential limitation of our study is that documenting symptoms of PCOS at age 31 and PCOS diagnosis at age 46 was based on questionnaires. Hirsutism might be over-reported by self-estimation and ovarian ultrasonography was not available to aid the diagnosis of PCOS. However, the combined presence of oligo/amenorrhea and hirsutism fulfils both Rotterdam and National Institute of Health (NIH) criteria for PCOS, and we have previously shown that co-existence of self-reported oligo-amenorrhea and hirsutism can identify women with the typical endocrine, metabolic and psychological profiles of PCOS (Taponen, Martikainen et al. 2003, Taponen, Ahonkallio et al. 2004),(Karjula, Morin-Papunen et al. 2017). It was not possible to differentiate between the diagnosis of chronic hypertension, PIH and preeclampsia, as all these conditions were included as one identity into the HDP diagnosis. Finally, even though BMI values were collected at ages 31 and 46 from clinical examination and postal questionnaire, BMI data during pregnancy were not available.

Conclusions

Polycystic ovary syndrome *per se* was not associated with an increased risk of HDP, but there was a strong synergistic association between PCOS, obesity and weight gain for the development of this pregnancy complication. The present results emphasize the importance of weight management prior pregnancy to reduce the incidence of HDP, an important factor cause of postnatal morbidity. The role of hyperandrogenemia in the pathophysiological process of HDP could not be confirmed, remaining under debate with further research being needed to clarify its role.

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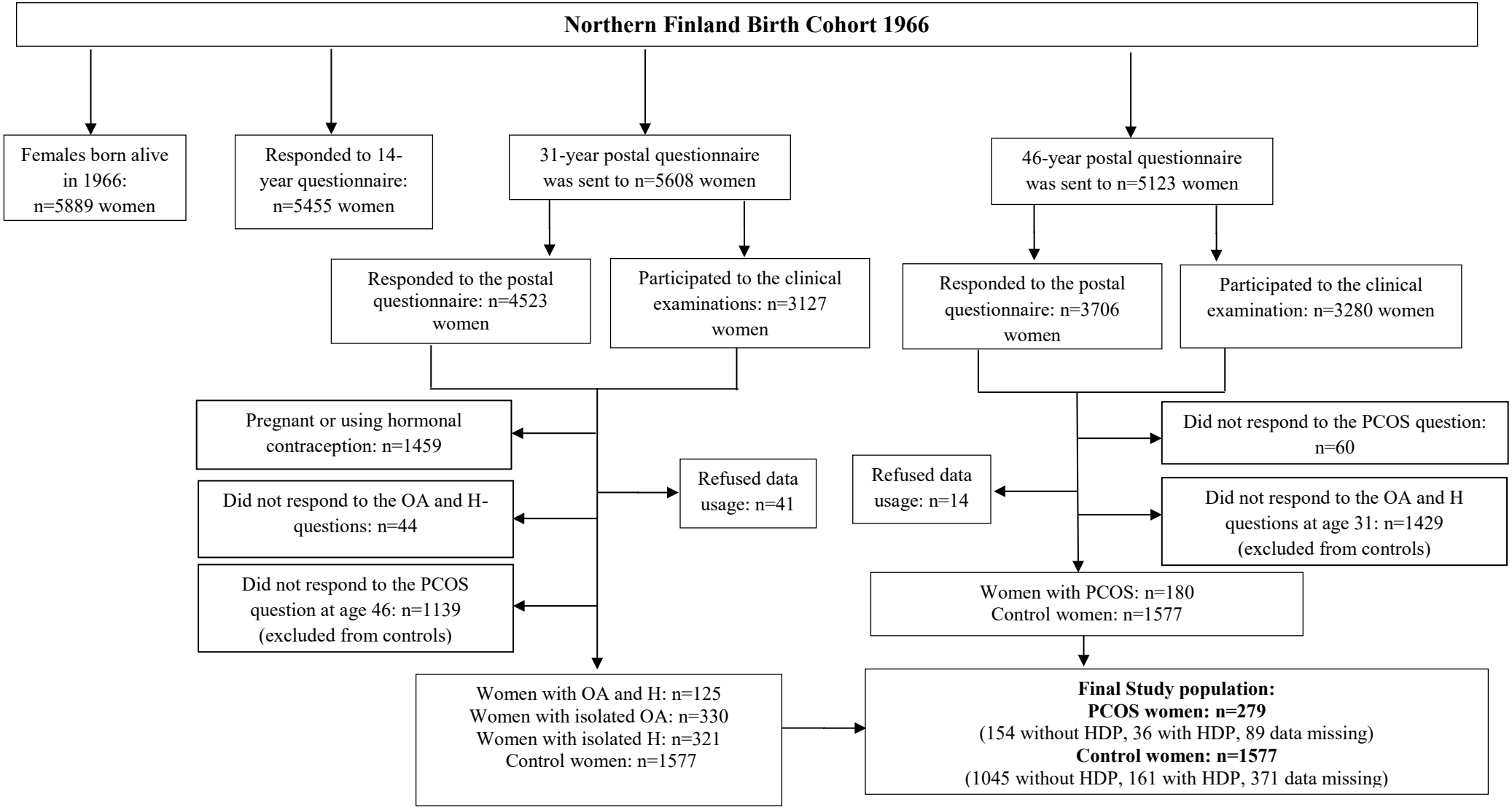
Table 1 Clinical characteristics of non-PCOS women and women with PCOS, sorted according to status of hypertensive disorder of pregnancy.

Clinical Characteristics	**Non-PCOS without HDP (n=521-1039[d])**	**Non-PCOS with HDP (n=85-160[d])**	**P-value[a] (BMI-adj. p-value[f])**	**PCOS without HDP (n=88-147[d])**	**P-value[b] (BMI-adj. p-value[f])**	**PCOS with HDP (n=21-35[d])**	**P-value[c] (BMI-adj. p-value[f])**	**P-value[d] (BMI-adj. p-value[f])**	**P-value[e] (BMI-adj. p-value[f])**
BMI (kg/m^2) 14 yr	18.7 (17.4; 20.2)	19.2 (17.9; 20.9)	.011	19.3 (18.0; 20.6)	.006	20.4 (18.6; 22.8)	<.001	.022	.018
BMI (kg/m^2) 31 yr	22.6 (20.7; 24.9)	24.0 (21.8; 26.7)	<.001	24.1 (21.5; 27.2)	<.001	28.0 (22.4; 33.9)	<.001	.001	<.001
BMI (kg/m^2) 46 yr	25.2 (22.6; 28.6)	26.9 (23.9; 30.5)	<.001	26.2 (23.7; 30.3)	.002	31.4 (26.1; 37.3)	<.001[t]	<.001[t]	.003[t]
Waist (cm) 31 yr	76.0 (70.0; 83.0)	78.0 (72.5; 85.3)	.005	81.0 (71.0; 92.0)	<.001	83.5 (75.0; 98.0)	<.001	.113	.021
Waist (cm) 46 yr	83.5 (77.0; 93.5)	90.0 (79.5; 99.0)	<.001	86.5 (79.5; 97.8)	.020	94.5 (83.6; 115.1)	.001[t]	.003[t]	.011[t]
Change in BMI (kg/m^2) 14-31 yr	3.83 (2.27; 5.74)	4.89 (3.21; 7.57)	<.001	4.59 (2.40; 7.54)	.009	5.94 (3.69; 11.1)	<.001	.015	.033
Change in BMI (kg/m^2) 31-46 yr	2.40 (0.81; 4.19)	2.65 (0.97; 5.61)	.068	2.19 (0.71; 4.60)	.745	2.96 (0.14; 4.40)	.895	.801	.479
Change in BMI (kg/m^2) 14-46 yr	6.33 (3.90; 9.33)	7.54 (5.32; 11.62)	<.001	7.21 (4.16; 10.5)	.151	9.82 (6.23; 14.6)	<.001[t]	.001[t]	.073[t]
Change in waist (cm) 31-46 yr	8.30 (2.60; 14.0)	8.80 (3.00; 16.0)	.352	8.00 (3.00; 15.0)	.692	11.0 (5.00; 19.9)	.203[t]	.247[t]	.691[t]
Total testosterone (nmol/l) 31 yr	1.80 (1.40; 2.30)	1.90 (1.40; 2.40)	.527 (.979)	2.00 (1.70; 2.80)	<.001 (.003)	2.60 (1.95; 3.10)	.002[t] (.083)	.284[t] (.558)	.011[t] (.061)
FAI 31 yr	3.75 (2.57; 5.44)	4.41 (2.67; 6.00)	.097 (.985)	5.08 (3.14; 7.64)	.002 (.372)	10.31 (4.55; 13.31)	.001[t] (.013)	.015[t] (.189)	.002[t] (.012)
Total testosterone (nmol/l) 46 yr	0.82 (0.62; 1.05)	0.80 (0.61; 1.07)	.963 (.664)	0.86 (0.68; 1.05)	.277 (.236)	0.97 (0.72; 1.13)	.092[t] (.066)	.315[t] (.232)	.113[t] (.063)
FAI 46 yr	1.53 (1.06; 2.22)	1.63 (1.17; 2.24)	.324 (.727)	1.62 (1.29; 2.25)	.064 (.347)	2.17 (1.43; 3.41)	.001 (.037)	.020 (.176)	.007 (.037)

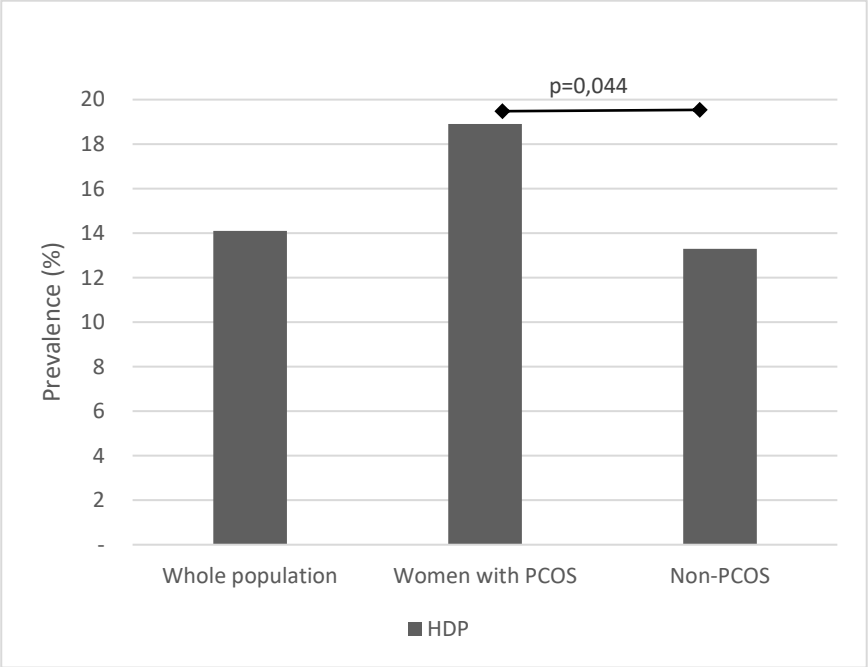
Data is expressed as medians (interquartile ranges). The significance test used was Mann-Whitney U test, when a rule |skewness/standard error of skewness|>1.96 was valid for the dependent variable, otherwise t-test was used ([t]-sign). [a]P-value: non-PCOS with HDP compared to non-PCOS without HDP. [b]P-value: PCOS without HDP compared to non-PCOS without HDP. [c]P-value: PCOS with HDP compared to non-PCOS without HDP. [d]P-value PCOS with HDP compared to PCOS without HDP. [e]P-value PCOS with HDP compared to non-PCOS with HDP. [f]The numbers of women in separate analyses varies due to non-response to some items. [f]The results were adjusted for BMI at age 31 and 46 years using univariate general linear modelling (ANCOVA). BMI: body mass index; FAI: free androgen index; HDP: hypertensive disorder of pregnancy; PCOS: polycystic ovary syndrome; SHBG: sex hormone binding globulin.

Figure 1. Flowchart of the study population. Data on pregnancies, deliveries and HDP until the end of 2013 was obtained from the questionnaire at age 46, the Finnish Medical Birth Register (FMBR) and the Hospital Discharge Register (HDR).



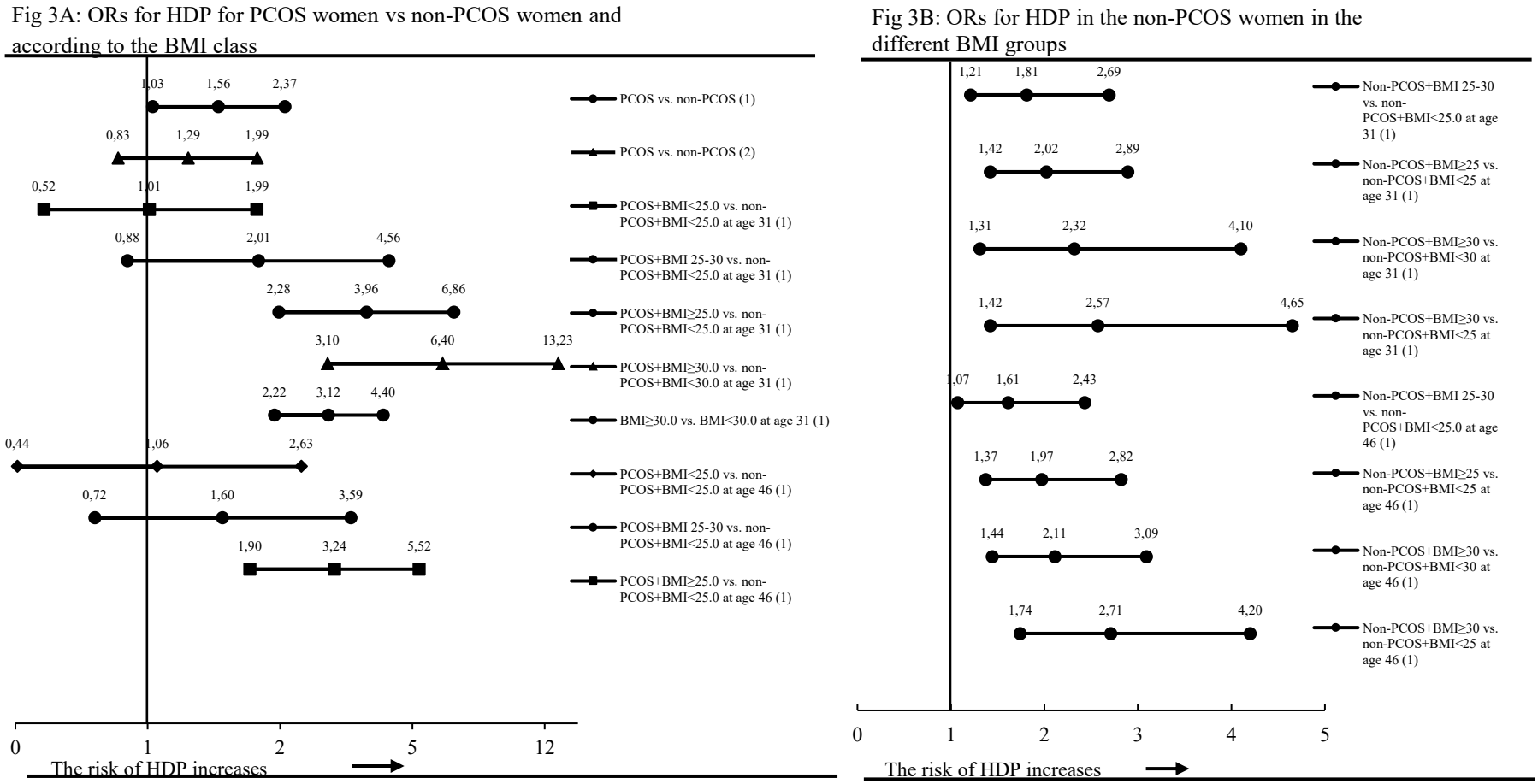
FMBR: Finnish Medical Birth Register; H: hirsutism; HDP: hypertensive disorder of pregnancy; HDR: hospital discharge register; OA: oligo-amenorrhea; PCOS: polycystic ovary syndrome.

Figure 2. Prevalence of HDP in the whole population (n=197/1396, and in women with (n=36/190) and without PCOS (n=161/1206).



HDP: hypertensive disorder of pregnancy; PCOS: polycystic ovary syndrome.

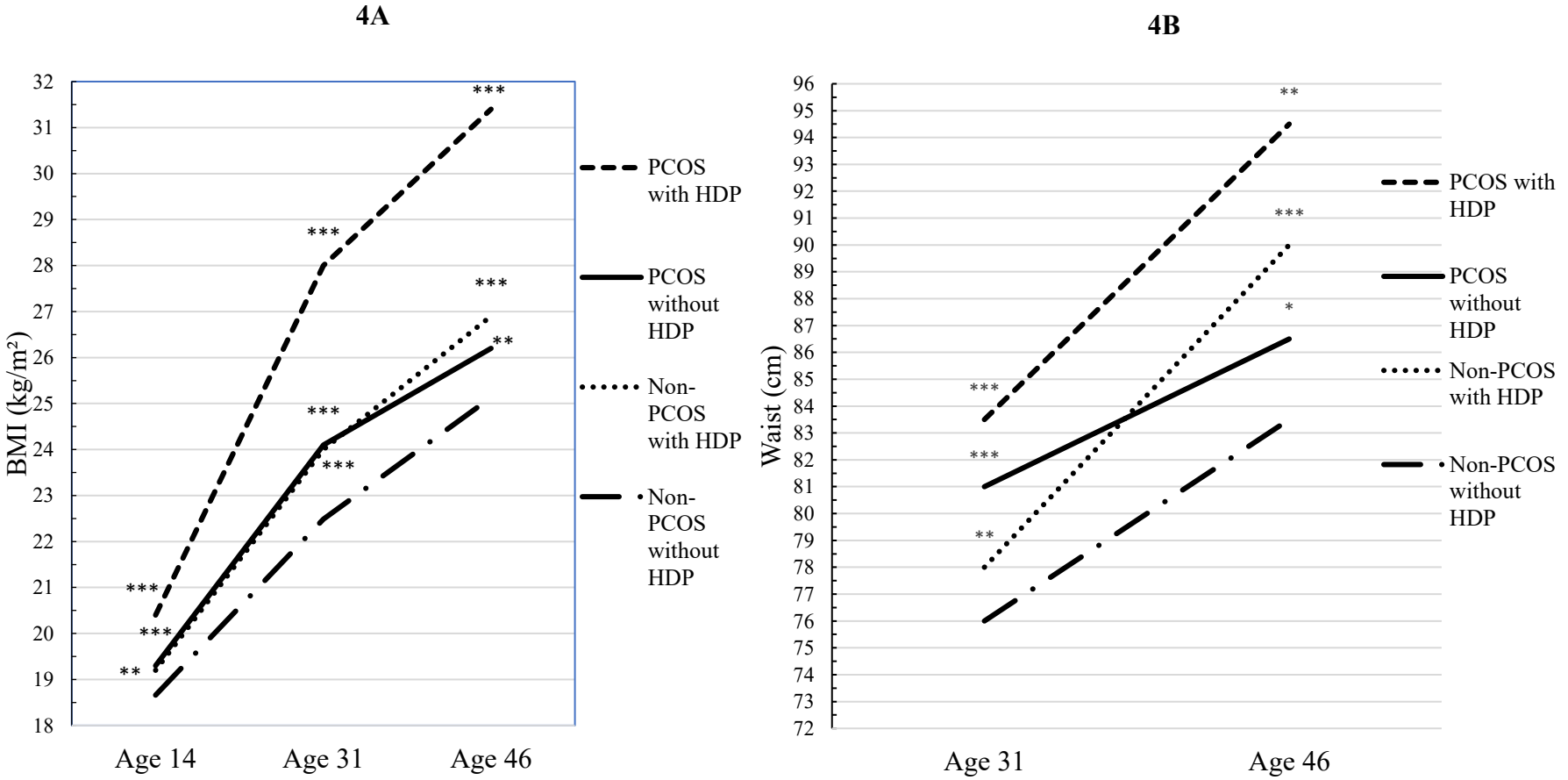
Figures 3A and 3B. Odds ratios (ORs) for hypertensive disorders of pregnancy.



The analyses were done using binary logistic regression. Results were adjusted model 1 (1) for consumption of alcohol, smoking and education or model 2 (2) for consumption of alcohol, smoking, education and BMI at age 31. BMI: Body mass index; CI: confidence interval; HDP: hypertensive disorder of pregnancy; T: total testosterone.

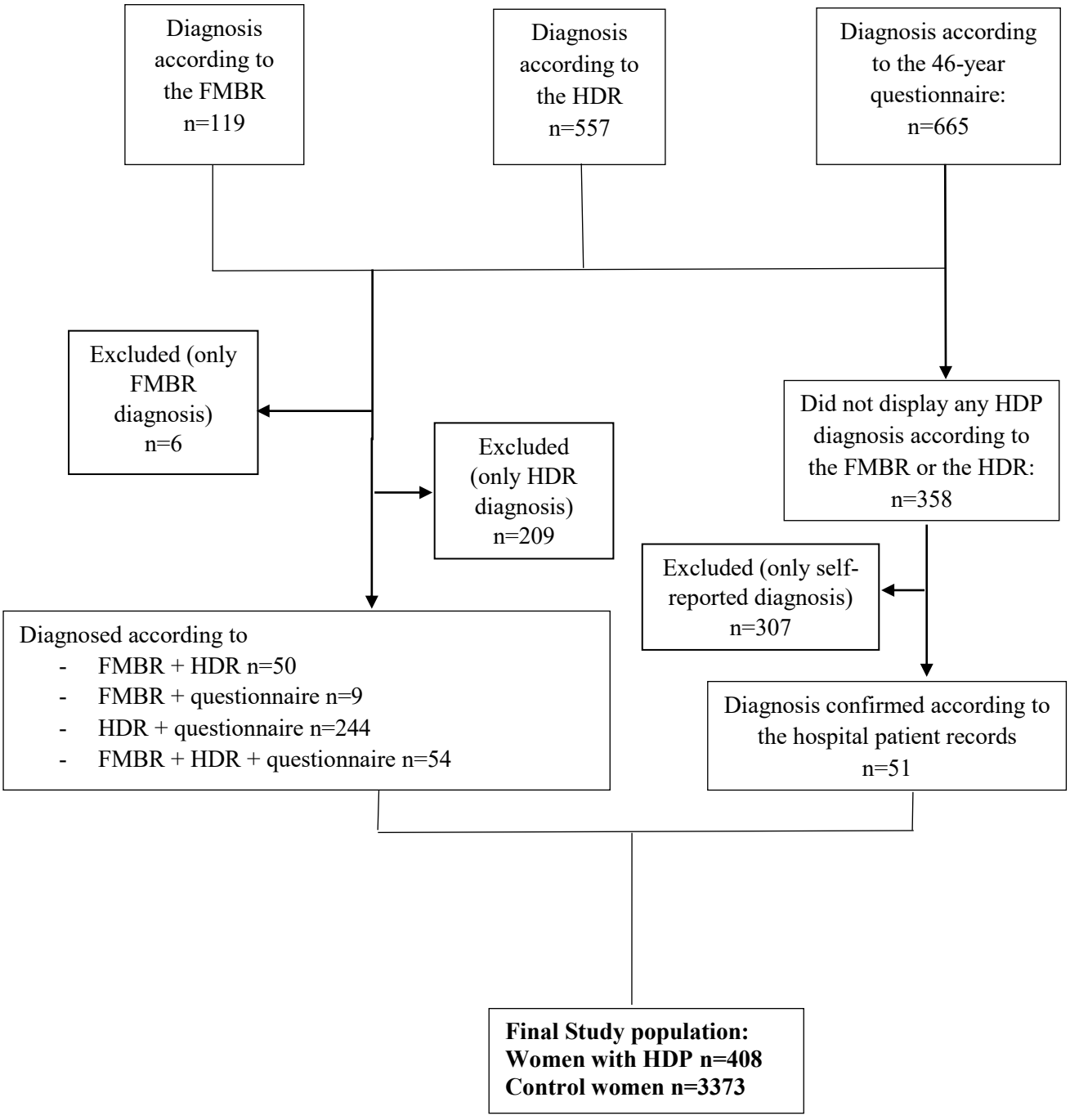
Figure 4A. Changes in body mass index (BMI) during life in women with PCOS and non-PCOS women according to their HDP status.

Figure 4B. Changes in waist circumference (cm) between ages 31 and 46 in women with PCOS and non-PCOS women according to their HDP status.

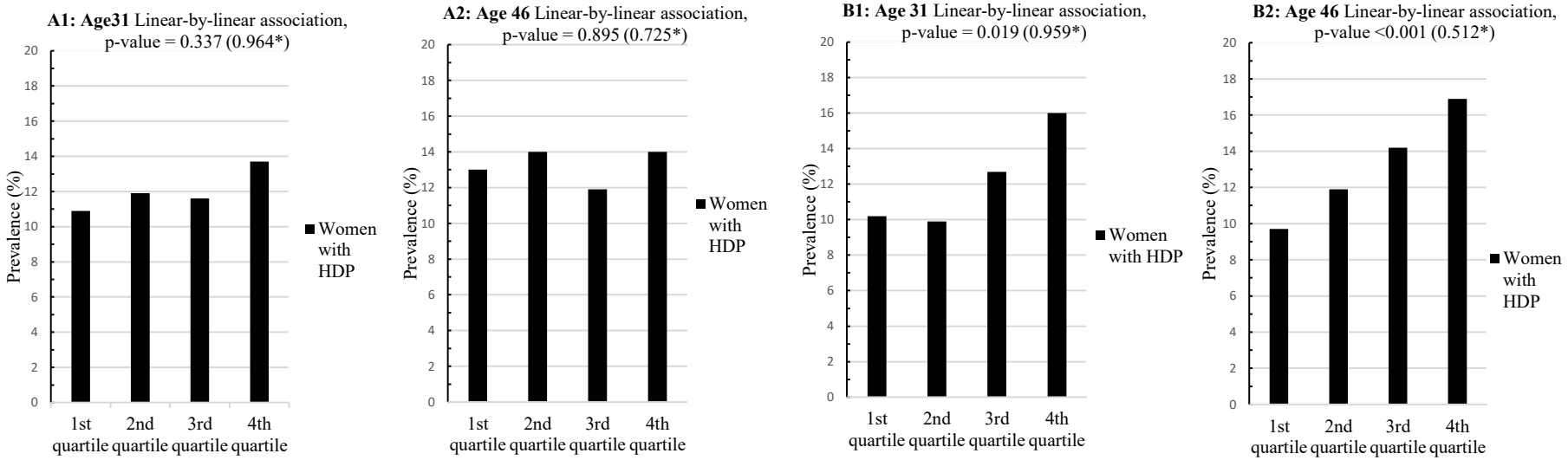


BMI and waist circumference values are expressed as medians at each time point (14, 31 and 46 years). The difference in the BMI and waist development between ages 14 and 46 in each study group was analyzed using the Mann-Whitney U test or t-test.
Only the P-values for comparison with non-PCOS women without HDP at the same age are presented here. The P-values for differences between the other groups are presented in the text and in Table 1. Figures 4A and B: *p < 0.05, **p < 0.01 and ***p < 0.001.
BMI: body mass index; HDP: hypertensive disorder of pregnancy; PCOS: polycystic ovary syndrome.

Supplemental figure 1. Flowchart of the HDP diagnosis. Data on HDP until the end of 2013 was combined from the questionnaire at age 46, the Finnish Medical Birth Register (FMBR) and the Hospital Discharge Register (HDR). The diagnosis for the final study population was assessed if at least two out of three sources was validated.



Supplemental figure 2A and 2B. Prevalence of hypertensive disorders of pregnancy (HDP) in different total level of testosterone (A) and free androgen (B) quartiles at ages 31 and 46.



The difference in the prevalence was analyzed using Chi-squared (χ^2) test's Linear-by-Linear association test to identify the trend of HDP prevalence across quartiles. The P-values represent the linear relationship in prevalence of HDP across the testosterone quartiles. *The overall BMI adjusted p-value was analyzed using binary logistic regression model. There were 418 subjects per testosterone and 417-418 subjects per FAI quartile at age 31 and 806 subjects per testosterone and FAI quartile at age 46. FAI: free androgen index. HDP: hypertensive disorders of pregnancy.